YEAST ARRAYS, METHODS OF MAKING SUCH ARRAYS, AND METHODS OF ANALYZING SUCH ARRAYS

FIELD OF THE INVENTION

The present invention relates generally to genomics and proteomics. More specifically, it relates to high density output arrays of multiple yeast strains, methods of making the high density output arrays, and methods of using the high density output arrays for functional analysis of genetic and protein-protein interactions.

BACKGROUND OF THE INVENTION

One of the major goals of the emerging field of proteomics is the establishment of relationships between protein function and particular diseases. Proteomic technologies are used to try to identify important genes and their related proteins implicated in diseases and their treatments and to understand the role these genes and their related proteins play in the onset and progression of disease. A major proteomics challenge is to determine the set of proteins expressed in the cell and the interactions between such proteins, which in turn define the functional pathways of the cell. If a given pathway is linked to a disease, then the proteins within the pathway or a functionally related pathway may represent drug targets for treatment of the disease.

Accordingly, there is a need in the art for functional proteomics technologies which provide valuable functional information about genes encoding proteins with previously unknown roles. Yeast based proteomics represents one such technology. Functional genomics and proteomics strategies involving large-scale construction of defined mutants have created the potential for the systematic mapping of genetic interactions on a genome-wide scale. In addition to the recent sequencing of the human genome, the genomes of other, simpler, organisms have been completely sequenced, including that of the budding yeast *Saccharomyces cerevesiae*. For *S. cerevisiae*, deletion mutations have been constructed for all 6,200 suspected genes, identifying a set of approximately 1200 essential yeast genes and approximately 5,000 nonessential genes, resulting in approximately 5000 viable haploid gene deletion mutants. With genome sequence in

hand, the monumental challenge is to understand the roles of the approximately 6,200 predicted yeast gene products. The scope of the challenge is immense. Approximately one-third of all predicted yeast genes are classified as coding for proteins of unknown function [Saccharomyces Genome Database (http://genome-www.stanford.edu/Saccharomyces/)]. Further, among a test pool of 558 homozygous deletion strains, over 60% had no observable growth defect after 60 generations. Simple extrapolation to more complex genomes suggests that the absence of obvious functions for a large fraction of encoded proteins will quickly become an enormous problem in biology.

There is a need, therefore, for proteomics technologies which can assess the previously unknown functions of proteins. The phenotypic analysis of the set of viable deletion strains within certain species of yeast represents a major challenge because the role of many genes will only be manifest under very specialized growth conditions. To address this problem, the present invention provides a high throughput method for the construction of yeast double-mutants that enables the phenotype associated with a specific mutation to be examined systematically within the context of thousands of different gene deletion backgrounds. A comprehensive application of this method will identify the precise genetic conditions under which each yeast gene is critical for fitness of the organism and may reveal a conserved network of genetic interactions linking fundamental processes and pathways of eukaryotic cells. Because many non-essential yeast genes have mammalian homologues systematic synthetic lethal analysis on yeast will provide crucial insights into the gene function problem in all eukaryotes. Such synthetic lethal analysis can be performed on the yeast arrays of the present invention. The high density yeast output arrays and the methods of analyzing such arrays of the present invention therefore fulfill a need in the art by providing simple and efficient methods for large-scale, high throughput analysis of genetic and protein-protein interactions.

SUMMARY OF THE INVENTION

The invention is directed to compositions and methods for performing large-scale analysis of genetic and protein interactions.

In one embodiment of the invention a high-density output array of multiple resulting yeast strains is constructed. Each resulting yeast strain in the output array contains at least one resulting genetic alteration different from the genetic alterations in the other resulting yeast strains in the output array.

5   The resulting yeast strains in the output array are mating products of at least two input arrays. At least one of the input arrays comprises multiple starting strains of yeast, each carrying at least one genetic alteration, with the genetic alteration being different in each starting yeast strain. The starting and resulting yeast strains are selected from any yeast strain that has two mating types and is capable of mating and meiotic and mitotic reproduction. Examples of 10   species that have such strains are *Saccharomyces cerevesiae* and *Schizosaccharomyces pombe*.

The input and output arrays are arranged on plates, with between about 96 and about 6144 yeast colonies on one plate, and much higher densities, over 10-fold higher, can be achieved if individual colonies are pooled. The resulting strains in the output array are double mutants. Two different types of output arrays are created, one in which the phenotypes 15   associated with mutations (genetic alterations) are examined within a diploid cell formed by mating the strains on the input arrays and another in which mutations are examined within the context of a haploid cell following sporulation of the diploids. The two mutations can involve a mutation of two different endogenous yeast genes. These yeast genes can be non-essential yeast genes. Interactions between deleted genes in the output array can be discerned when the 20   combination of genes leads to either a synthetic lethal double mutant or a synthetic sick double mutant, i.e. where the double mutant grows more slowly than either of the single mutants on the input arrays. The entire output arrays can comprise between about 1,000 and about 25 million resulting strains of yeast, or between about 1 million and about 25 million resulting yeast strains.

The input array contains starting yeast strains with starting genetic alterations in at least 25   one starting yeast strain. The genetic alterations can be of any of the following type: (i) an alteration in the DNA encoding the gene such as a deletion or mutation of an endogenous essential or non-essential yeast gene; (ii) *trans*-dominant genetic agents such as genes coding for nucleic acid or peptide aptamers, dominant-negative proteins, antibodies, small molecules, natural products, ribozymes, enzymes, RNAi, and antisense RNA or DNA; (iii) protein and 30   RNA expression vectors of a heterologous gene from a viral, prokaryotic, or eukaryotic genome,

wherein the genes can either be wild type, mutated or fragmented (*e.g.* coding for a protein domain); (iv) a protein-protein interaction detection system, including expression plasmids coding for a two-hybrid interaction and reporter that registers the interaction, the Ras recruitment system, the split-ubiquitin system, and various other protein fragment complementation systems (*e.g.* DHR); and (v) a reporter whose expression reflects a change in cellular state such as the activation or the inhibition of a pathway(s). The genetic alterations can be integrated into the yeast genome or propagated on autonomously replicating plasmids.

The aptamer can be either a peptide aptamer or a nucleic acid aptamer. It can either inhibit or enhance expression of genes, protein interactions, or the activity of a protein or any other cellular component.

The heterologous gene can be a human gene that can be a single nucleotide polymorphism of another human gene.

In another embodiment of the invention, a high-density output array of resulting multiple yeast strains, where each resulting yeast strain carries at least one resulting genetic alteration, and the resulting genetic alterations are different in each yeast strain, is constructed through the method disclosed below. Multiple starting yeast strains are generated, each strain carrying a starting genetic alterations. Sets of two starting yeast strains, each of the two sets containing a different starting genetic alteration; are then mated. The mated strains are then made to undergo sporulation, resulting in haploid spore progeny. A single mating type is then germinated and the haploid spore progeny is cultured using selective growth criteria. Multiple haploid yeast strains which carry a resulting genetic alteration which is a combination of at least two starting genetic alterations are selected for through this process. The genetically altered yeast strains are then arrayed in a high-density format on an output array.

The strains, plates, and genetic alterations used in this embodiment are similar to the previous embodiment. The output array described in this embodiment could also be used to perform synthetic lethal analysis as described in the previous embodiment.

Yet another embodiment of the invention is a method for conducting small molecule screening of yeast colonies using a high-density input array of multiple starting yeast strains. The method is carried out by generating an input array containing multiple starting yeast strains as

described above. Then exposing this array to at least one biological effector, and detecting change in phenotype in the starting strains in response to the effector. The effector can be a small molecule or any other biological effector. The input array and genetic alterations can be the same array described in previous embodiments.

5   Yet another embodiment of the invention is a method for conducting synthetic lethal analysis of yeast colonies by producing an input array of starting strains, and crossing the starting strains in that array with other starting strains or another input array. Then the diploid resultant strains are studied for changes in phenotype due to the combination of different genetic or chemical alterations.

10   The input arrays are constructed as detailed in the embodiment above. The genetic alterations can be the same as the ones in the embodiments above. Chemical alterations can include biological effectors such as the ones described in the previous embodiment.

Yet another embodiment of the invention is a method to perform synthetic lethal analysis of yeast colonies of multiple yeast strains using DNA bar coding. In this method, starting strains are constructed as described above, but not placed into arrays. Each of the genetic alterations has a distinct DNA tag associated with it. This tag can be a 20 nucleotide long DNA sequence associated with a certain genetic alteration. These starting strains are mated with other starting strains of a different mating type, which generates the first output strain set, and then stimulated to undergo sporulation, which allows for selected growth of haploid spore progeny that possess of both genetic alterations and generates the second output strain set. The resulting output strains are then studied and isolated through their genetic tags, dispensing with the need to array each strain. The genetic alterations used in this embodiment can be the same alterations used in the previous embodiments.

25   **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 illustrates the series of replica pinning procedures in which mating and meiotic recombination are used to generate haploid double mutants.

Figure 2A illustrates the array of *bni1*Δ double mutants resulting from the final pinning and the corresponding wild-type control.

Figure 2B illustrates that both the *bni1* Δ *bnr1* Δ and *bni1* Δ *cla4* Δ double-mutants were

5   inviable and that the *bni1* Δ *bud6* Δ double-mutant was associated with a slower growth rate or "synthetic sick" phenotype, reflecting reduced fitness of the double-mutant relative to the respective single mutants.

Figure 3 illustrates a genetic network constructed from the interactions listed in Table 1.

10   A genetic interaction network representing the synthetic lethal/sick interactions is determined by the synthetic genetic analysis of the present invention. Genes are represented as nodes and interactions are represented as edges that connect the nodes, 292 interactions and 205 genes are shown. The genes are colored according to their YPD cellular roles, with the most abundant cellular roles shown.

15   Figure 4 illustrates two dimensional clustering analysis of synthetic lethal interactions. The set of synthetic lethal interactions associated with mutations in 8 query genes, *BIM1*, *BNI1*, *ARC40*, *ARP2*, *NBP2*, *BBC1*, *RAD27* and *SGS1*, were plotted on the horizontal axis, with the query gene cluster tree above. The 201 genes that showed synthetic lethal interactions with the

20   query genes were plotted on the vertical axis with the cluster tree on the leftmost side of the plot. Synthetic lethal and synthetic sick (slow growth) interactions are represented as shades of red. An expansion of the plot is shown to allow visualization of specific genes.

25   **DETAILED DESCRIPTION**

The present invention is directed to high-density output arrays of multiple yeast strains, methods for constructing such arrays, and methods of using such arrays to conduct large-scale high throughput analysis of genetic and protein-protein interactions. The present invention provides a systematic and efficient method for constructing arrays of yeast strains carrying

30   multiple genetic alterations. This invention enables a large-scale analysis of genetic and protein-

protein interactions, provides a method for validating potential drug targets and for generating whole cell screens for compounds that perturb the function of these targets.

5   More specifically, the present invention provides compositions and methods that will enable the systematic and automated construction of arrays of yeast strains containing multiple mutations in a high-throughput manner. For example, the present invention could be used to generate a high density output array of approximately 25 million double mutant yeast strains, through crosses of input arrays involving approximately 5,000 viable haploid deletion mutants. The resulting output arrays can be used for large-scale analysis of genetic and protein interaction networks by identifying the complete set of synthetic lethal double mutant combinations for a

10   model eukaryotic cell.

     The invention is directed to a variety of high-density yeast arrays, methods of making such arrays, and methods of using such arrays. The arrays include both input and output arrays. Input arrays are those arrays used to generate output arrays. Input arrays are arrays of multiple yeast strains, with each yeast strain containing at least one genetic alteration. These input arrays

15   are crossed with either other starting yeast strains that contain at least one genetic alteration, or other input arrays to produce output arrays. Output arrays are therefore generated by crossing starting yeast strains from input arrays. The starting yeast strains each have at least one genetic alteration. Crossing of the starting yeast strains containing at least one genetic alteration results in strains of the output arrays containing at least two genetic alterations. These genetic

20   alterations present in both the input and output arrays can include, but are not limited to, members of the group consisting of an aptamer, a system for detecting protein-protein interactions, including a yeast two-hybrid system, expression of a heterologous gene from a viral, prokaryotic, or eukaryotic genome with the heterologous gene either having or not having a yeast homolog, transformation with a promoter operably linked to a reference gene, wherein the

25   reference gene can be a reporter gene, mutation or deletion of an endogenous essential or non-essential yeast gene, or the addition of other dominant agents which can perturb any cellular function, including genes coding for dominant negative proteins, antibodies, small molecules, natural products, ribozymes, RNAi, and antisense RNA or DNA.

     As used herein, an input array is a grouping of a multitude of starting yeast strains located together on a solid support. In one embodiment, the solid support is a plate. In a preferred

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embodiment, there are between about 96 and 6144 colonies on one plate, preferably the array would be constructed at the highest density physically possible, which is limited by cell size and the number of genetically different strains that can be pooled and manipulated as one element of the array. The starting strains in the input array are selected from any yeast species that has two

5 mating types and is capable of meiotic and mitotic reproduction. In preferred embodiments, the starting yeast strains can be from either the *Saccharomyces cerevesiae* species or the *Schizosaccharomyces pombe* species.

As discussed above, the genome of *S. cerevisiae* has been completely sequenced, and approximately 6,200 genes have been located. The international *S. cerevisiae* deletion

10 consortium has constructed mutants corresponding to all of the approximately 6,200 suspected genes within the *S. cerevisiae* genome, with each mutant being a strain of yeast containing a deletion of a different endogenous yeast gene. Tetrad analysis has revealed that approximately 15% of these deletion mutations define genes essential for the viability of haploid cells, also referred to as "essential genes". Thus, the deletion consortium identified approximately 1,200

15 essential genes and approximately 5,000 nonessential genes.

The set of approximately 1,200 essential genes does not define the minimal set of genes required for life because many genes that are individually dispensable are not simultaneously dispensable. Synthetic genetic interactions are usually identified when a specific mutant is screened for second-site mutations that either suppress or enhance the original phenotype. In

20 particular, two genes show a "synthetically lethal" interaction if they are associated with viability as single mutations but combine to cause a lethal double-mutant phenotype. This phenotype in which a particular gene is only required for cell viability when another gene is also deleted is called a "synthetic lethality defect". Similarly, the deletion of some genes results in a more severe growth defect only when combined with the deletion of another gene, a phenotype

25 referred to herein as a "synthetic fitness defect". Because both synthetic lethal defects and synthetic fitness defects represent genetic interactions, the reference herein to synthetic lethal interactions or relationships also applies to synthetic fitness interactions.

Many synthetic lethal relationships appear to be specific, occurring for genes acting on a single biochemical pathway. They also occur, however, for genes within two distinct pathways

30 if one process functionally compensates for defects in the other or if the two processes are

functionally related. Genetic screens for synthetic lethal interactions traditionally involve three labor-intensive steps: (1) mutagenesis of a strain that carries a mutation in a "query" gene of interest; (2) isolation of mutants whose growth is dependent upon expression of the query gene; and (3) cloning of the synthetically lethal gene by complementation with a plasmid-born

5   genomic library. Synthetic lethal screens have been applied successfully to identify genes involved in cell polarity, secretion, DNA repair, and numerous other processes. On average, three to four different interactions were identified per screen, but very few of the published screens approach saturation.

With a collection of defined mutations, synthetic lethal interactions can also be identified

10   through systematic construction and analysis of double-mutants. The identification of synthetic lethal/fitness double-mutant combinations often indicates significant *in vivo* interactions between gene products and serves as a key starting point for targeted biochemical or cell biological experiments. Indeed, a synthetic lethal/fitness mutant combination is often observed for genes that impinge on the same essential cellular function. As described above, because the genome of

15   *S. cerevesiae* is sequenced and most of the essential and non-essential genes are determined, yeast arrays crossing strains with deletions or mutations of non-essential genes can be generated in order to study the effects of double mutations. Accordingly, *S. cerevesiae* is an organism which works well in the high-density output yeast arrays of the present invention.

Additionally, the same methods described herein for the *S. cerevisiae* genome wide

20   deletion set can be applied to other yeasts once analogous deletion sets are constructed. In particular, the fission yeast *Schizosaccharomyces pombe* has proven an invaluable complementary organism to budding yeast for cell and molecular genetic analysis. The completion of the fission yeast genome presents an enormous opportunity for comparative biology between the two distantly related yeasts. Within the next several years, a complete gene

25   deletion set will certainly be constructed by the *S. pombe* community, and would be easily amenable to the systematic synthetic lethal analysis using the large-scale genetic and protein interaction analysis methods of the present invention. Any other fungal species that has two mating types can also be used with the present invention.

As stated above, the input array of the present invention contains a multitude of starting

30   yeast strains. The array could contain, for example, about 5000 different yeast strains, each of

which contains a different gene deletion. Such an input array could be crossed with a second

input array, which also contains about 5000 different yeast strains, each of which also contains a

gene deletion. This type of output array containing approximately 25 million (5,000 x 5,000)

different yeast strains is described in detail in Example 5. Or, in another embodiment, an input

5   array of about 5000 different yeast strains could be crossed with only one starting strain.

Examples of these types of crosses are described in detail in Example 6. In yet another

embodiment, more specific input arrays can be crossed with specific deletion mutants, as

illustrated in Examples 7-10. The possibilities of different crosses between a first input array and

a second input array or between a first input array and a starting strain are quite numerous, and

10   will be discussed in more detail below.

In one embodiment of the invention, a first input array contains yeast strains, which are

modified so that they contain at least one non-lethal genetic alteration. The first input array is

then crossed with either another yeast starting strain or another input array, which also contains

at least one genetic alteration, to form an output array containing double mutants. Double

15   mutants as used herein means yeast strains which contain two genetic alterations, derived from

single mutants each of which contains at least one genetic alteration.

An output array is the product of a cross between either two input arrays or an input array

and a starting strain. The input arrays and/or starting strain to be crossed are of different mating

type, to allow for selection of the genetic alterations formed after the cross. For high-throughput

20   synthetic lethal analysis with the set of viable yeast gene deletion mutants, the present invention

provides a series of replica pinning procedures in which mating and meiotic recombination are

used to generate two output arrays, one composed of diploid cells carrying genetic alterations

derived from the input arrays and another output array containing haploid meiotic progeny

carrying genetic alterations derived from the input arrays (Fig. 1). In this scheme, a query

25   mutation is first introduced into a haploid starting strain, of one mating type, and then crossed to

the array of gene deletion mutants of the opposite mating type. A set of diploids that are

heterozygous for both mutations represents the first output array that can be analyzed for a

phenotype. Sporulation of the diploid cells leads to the formation of double-mutant meiotic

progeny and the second output array. The starting strain carries a reporter that allows for selected

30   germination of spores, which ensures that conjugation of meiotic progeny does not complicate

the final analysis. Both the query mutation and the gene deletion mutations can be linked to dominant selectable markers, which enables selected growth of double-mutants specifically. The final pinning results in an ordered array of double-mutant strains, whose growth rate is monitored by visual inspection or image analysis of colony size.

5   The output arrays are generated by performing the following steps. The first step is to generate multiple starting yeasts strains, with each of these starting yeast strains carrying a genetic alteration. These starting yeast strains are then grouped into either two input arrays, or one input array and a particular starting strain. The input arrays are then crossed (mated), and diploid strains result, forming the first output array. The mated diploid strains then undergo

10   sporulation, resulting in haploid strains. A single mating type is germinated. The haploid spore progeny that result from this sporulation are then grown using selective growth criteria. Multiple haploid yeast strains, which grow on the selective media, can then be selected for the presence of genetic alterations that were in the starting strains. These recovered haploid yeast strains can then be arrayed in a high-density format forming the second output array.

15   output array can contain hundreds, thousands, or millions of resulting yeast strains with genetic alterations. In one embodiment, the output array contains between about 1,000 and 25 million resulting yeast strains, and more preferably between about one and about 25 million resulting yeast strains. Because the output array can be produced in such a high-density format, containing millions of yeast strains, the output array can be used to assign gene function to

20   multiple genes simultaneously. The high-density output array also allows for large-scale analysis of genetic and protein interactions, by analyzing the phenotypes of the resulting strains within the output array.

One type of analysis that can be performed on the high-density output arrays is a synthetic lethal/synthetic fitness analysis. Such an analysis is used to determine the presence or

25   absence of synthetic lethality defects or synthetic fitness defects. Synthetic lethality defect and synthetic fitness defect are phenotypes wherein either cell death or retarded cell growth occurs only when two different genes are deleted at the same time. When a specific gene is required for cell viability under conditions when a different gene is deleted or mutated, this resulting phenotype is termed a "synthetic lethality defect". This phenotype is so named because the two

30   genes deleted together synthetically lead to cell death. Similarly, the deletion of some genes

results in a more severe growth defect only when combined with the deletion of another gene, a phenotype referred to as a "synthetic fitness defect". Experience suggests that a fraction of the genes of unknown function in *S. cerevisiae* will not exhibit synthetic lethal interactions with other single deletions. However, this defined subset represents the next logical target for a

5   subsequent round of synthetic lethal screens that would yield genes that are essential only when two other genes are deleted. A slight modification of the selection scheme will allow the synthetic lethal screens to be reiterated. By definition, when taken to the limit, this approach will identify the function of all genes encoded by the yeast genome.

The identification of synthetic lethal/fitness double-mutant combinations often indicates

10   significant functional interactions between gene products or the pathways containing the gene products and serves as a key starting point for targeted biochemical or cell biological experiments. Accordingly, when a first input array is crossed with either a second input array or a starting yeast strain, the phenotypes of the crosses are studied to see if the resulting strain develops a synthetic fitness, or synthetic lethal phenotype, or any other discernible phenotype. In

15   particular, the methods used in this invention include the use of groups of fungal strains that contain genetic alterations. These strains can be crossed in order to study the phenotype of a strain with at least two genetic alterations.

The genetic alterations can be of any of the following types: (i) an alteration in the DNA encoding the gene such as a deletion or mutation of an endogenous essential or non-essential

20   yeast gene; (ii) introduction of *trans*-dominant genetic agents such as genes coding for peptide or nucleic acid aptamers, dominant-negative proteins, antibodies, small molecules, natural products, nucleic acid aptamers, ribozymes, enzymes, RNAi, and antisense RNA or DNA; (iii) protein and RNA expression vectors of a heterologous gene from a viral, prokaryotic, or eukaryotic genome, wherein the genes can be either wild type, mutated, or fragmented (e.g. coding for a protein

25   domain); (iv) a protein-protein interaction detection system, including expression plasmids coding for a two-hybrid interaction and reporter that registers the interaction, the Ras recruitment system, the split-ubiquitin system, and various other protein fragment complementation systems (*e.g.* DHR); and (v) a reporter whose expression reflects a change in cellular state such as the activation or the inhibition of a pathway(s). The genetic alterations can be integrated into the

30   yeast genome or propagated on autonomously replicating plasmids.

The input arrays and starting yeast strains which are crossed to produce an output array can contain within them a variety of genetic alterations, which allow for analysis of a variety of different modifications. In one embodiment of the invention, the yeast starting strains in the input arrays have a gene deletion introduced into them as their genetic alteration. This gene deletion could be of an essential, or non-essential gene. Non-essential gene deletions are deletions or mutations of genes that do not produce a lethal phenotype. In *Saccharomyces cerevesiae,* there are approximately 5,000 non-essential genes which, when either deleted or mutated, do not produce a lethal phenotype. Combinations of non-essential genes deletions can produce synthetic lethal or synthetic fitness phenotypes that will reveal how these genes interact, what their function is in yeast i, and what the function of their homologs are in humans or other organisms. Combinations of these genes could also lead to other discernible phenotypes, which could suggest the function of the deleted genes.

Essential genes are genes that when deleted or mutated cause a lethal phenotype. The function of essential genes can be studied by modulating their expression using inducible promoters. Similarly, conditional mutations can be introduced into essential genes allowing the mutant phenotype to be analyzed under defined conditions. For example, temperature sensitive mutations are viable at a permissive temperature and inviable at a restrictive temperature.

In one embodiment of the invention, an input array with a non-essential or essential gene deletion can then be crossed with other starting strains that contain other gene deletions, or any other genetic alteration. Genetic alterations mentioned in regards to this invention may include: non-essential gene deletions; essential gene deletions; aptamers; exogenous genes, either wild type, mutated, or fragmented (e.g. coding for a protein domain); genes coding for ribozymes; enzymes; RNAi, and antisense RNA or DNA; systems for detecting protein-protein interactions such as the yeast two-hybrid system; and reporters whose expression reflects changes in cellular state.

In another embodiment of the invention, yeast-starting strains in the input arrays have aptamers either integrated into their genome or introduced as expression plasmids as their genetic alteration. Aptamers are peptide or nucleic acids that are produced through at least partially randomized pools of nucleic acid or amino acid sequences, that are selected for their ability to bind certain epitopes. Peptide aptamers are defined as affinity agents that consist of

constrained combinatorial peptide libraries displayed on the surface of scaffold proteins. Peptide aptamers are trans-dominant agents that interact with gene products.

Ordered arrays of yeast strains expressing peptide or nucleic acid aptamers can substitute for arrays of yeast deletion strains. In this embodiment, starting strains containing gene deletions are crossed to an array of strains expressing peptide or nucleic acid aptamers and haploid meiotic progeny expressing the peptide aptamer and carrying the gene deletion can be selected. The resulting strains that show aptamer-dependent synthetic lethality identify aptamers that inhibit a gene product whose activity is required for viability of the starting gene deletion strain. The array of strains expressing peptide aptamers could also be used to identify dominant inhibitors. In this case, a strain carrying a query mutation would be crossed to an array of strains expressing peptide aptamers and the resultant diploid cells examined directly for a phenotype. Those that show aptamer-dependent synthetic lethality as diploids would identify an aptamer that inhibits a gene which shows a genetic interaction with the heterozygous query mutation.

Aptamers can inhibit the function of gene products by any one of, but not limited to only, the following mechanisms: (i) modulating the affinity of a protein-protein interaction; (ii) modulating the expression of a protein on a transcriptional level; (iii) modulating the expression of a protein on a post-transcriptional level; (iv) modulating the activity of a protein; and (v) modulating the location of a protein. The precise mechanism of action of peptide aptamers can be determined by biochemical and genetic means to ascertain their specific function in the context of their interaction with other genes, and gene products. Strains carrying characterized aptamers can then be crossed with other starting strains that contain peptide aptamers or any other genetic alteration as described above. The phenotypes of these crosses can then be studied to determine if the resulting strains in the output array develop a synthetic fitness, or synthetic lethal phenotype, or any other discernible phenotype.

In another embodiment of the invention, the starting strains would carry a heterologous gene or gene combination with a readout of gene product activity. For example, the starting strain may contain a heterologous gene encoding an enzyme for which there is a biochemical assay for its activity. In another example, the starting strain may carry a yeast two-hybrid protein-protein interaction system or some other protein-protein interaction detection system such as the Ras recruitment system, the split-ubiquitin system and various other protein fragment

complementation systems (e.g. DHR), which can be crossed to yeast within the input array that contain other yeast two-hybrid interaction systems, or any other genetic alteration. The genetic alterations in the other starting strain could be any of the ones defined above or any other genetic alterations. The phenotypes of these crosses can be studied to determine if any of the resulting

5   strains within the output array perturb the two-hybrid interaction. For example, if an input starting strain carries a set of genes that allow for the formation and detection of a two-hybrid interaction and the strains within the input array carry yeast gene deletions, then the output array would allow for the identification of deletions that perturb the two-hybrid interaction; or if the strains within the input array carry peptides aptamers, then this system can be used to identify

10   dominant inhibitory peptide aptamers that perturb the two-hybrid interaction.

In yet another embodiment of the invention, the starting strains in the input arrays can express a heterologous gene(s) from either a prokaryotic, viral, or eukaryotic genome. In a preferred embodiment, heterologous genes are from the human genome. Different alleles of these genes can be tested in yeast to see how they interact with mutated yeast genes that are

15   homologous to human genes. This method can be used to assign function to different exogenous genes. Single nucleotide polymorphisms (SNPs) of human genes could also be characterized to identify SNP-dependent interactions. The genes of any organism could be similarly manipulated with this system. These heterologous genes can replace their deleted yeast homolog, or they can be genes that are not homologous to any yeast gene. These exogenous genes could be crossed

20   with strains that contain either any of the genetic alterations described above, or any other genetic alteration.

Many yeast genes are conserved from yeast to humans and thus it is possible to functionally replace a yeast gene with its human homolog. There are many different alleles of a given human gene and some of these may be associated with a diseased state. The replacement

25   of a yeast gene by set of alleles of its human homologue, each differing by one or more SNP (single nucleotide polymorphism), in the context of the described genetic arrays offers a means to assess the functional interactions of a given allele within a model eukaryotic cell. For example, the analysis of different alleles of a human gene may reveal that one allele in particular is associated with a greater number of synthetic lethal interactions, which suggests it is

30   compromised for function relative to other alleles and, therefore, may be associated with a

diseased state. If more than one conserved human gene is implicated in a diseased state, then in theory all combinations of different alleles can be tested for function within the context of a genetic array.

Yeast genetic arrays also permit the functional analysis of heterologous genes that do not have yeast counterparts. For example, consider a human gene, designated *hXXX*, whose product is involved in reorganization of the actin cytoskeleton and for which there is no yeast counterpart. Even though yeast cells do not contain a homolog *hXXX*, yeast cells have a highly conserved actin cytoskeleton and therefore will likely contain gene products, such as actin, that the *hXXX* gene product may interact with. Thus, expression of the human gene within the context of a yeast genetic array will likely result in synthetic lethal/fitness defects that link the function of *hXXX* to actin reorganization. The heterologous gene could be taken from any viral, prokaryotic, or other eukaryotic genome.

Starting strains carrying heterologous genes can be crossed with starting strains containing another exogenous gene, or any other genetic alteration. The genetic alterations in the other starting strain could be any of the ones defined above or any other genetic alterations. The phenotypes of these crosses can then be studied to determine if the resulting strains in the output array develop a synthetic fitness, or synthetic lethal phenotype, or any other discernible phenotype.

In another embodiment of the invention, the starting strains in the input array contain a promoter from either a prokaryotic, viral, or eukaryotic genome operably linked to a reference gene. Starting strains that carry a promoter operably linked to a reporter gene can be crossed with starting strains containing another promoter and reporter gene, or any other genetic alteration. The genetic alterations in the other starting strain can be any of the ones defined above or any other genetic alterations. The phenotypes of these crosses can then be studied to determine if the resulting strains in the output array develop a synthetic fitness, or synthetic lethal phenotype, synthetic dosage lethality, expression of the reporter gene, or lack thereof, or any other discernible phenotype.

The ability to control the expression levels of genes with regulated promoters is especially useful for synthetic dosage lethality screens. Synthetic dosage lethality is a specialized version of a classical synthetic lethality screen. In synthetic dosage lethality, a

reference gene is overexpressed in set of mutant strains carrying potential target mutations. This reference gene can be an exogenous gene from a viral, prokaryotic, or eukaryotic genome. More specifically, the gene could be a human gene. Increasing the amount of the reference gene product may not produce a phenotype in a wild-type strain. However, a lethal phenotype may

5 result when overexpression of a gene product is combined with decreased activity of another gene product that impinges on the same essential function. For example, synthetic dosage lethality has been used to identify genetic interactions between *CTF3*, which encodes a centromere binding protein, and a set of conditional kinetichore mutants. The synthetic dosage lethality gene could also contain single nucleotide polymorphisms.

10 Genes can be overexpressed by cloning the open reading frame (ORF) behind a strong promoter, such as the galactose-induced *GAL1* promoter. Input arrays containing starting strains with the approximately 5,000 different yeast deletions can be crossed to a starting strain carrying a plasmid that contains a *GAL1*-regulated reference gene. Haploid meiotic progeny carrying *GAL1*-regulated reference gene and carrying the gene deletion can be selected. The output array

15 containing yeast deletions combined with the *GAL1*-regulated reference gene can be pinned from glucose medium, where the reference gene is not expressed, to galactose medium, where it is overexpressed, to score for synthetic dosage lethality.

In another embodiment of the invention, the gene-encoding reporter, such as green fluorescence protein (*GFP*), may be placed on a plasmid under the control of a regulated

20 promoter. One useful promoter is the pheromone-induced *FUS1* promoter, *FUS1pr*, which is massively induced by stimulation of the MAP-kinase pathway that mediates the pheromone response in yeast. A *FUS1pr-GFP* gene could be constructed and introduced into the set of yeast deletion mutants for expression analysis. Mutations that lead to increased basal levels of *FUS1pr-GFP* expression, i.e. levels above that displayed by wild-type cells, would identify

25 genes that encode potential negative regulators of the pheromone response signal transduction pathway. By contrast, mutations that lead to decreased basal levels of *FUS1pr-GFP* expression would identify genes that positively activate the activity of pheromone response pathway signaling molecules. Quantitative analysis of GFP expression can be achieved from agar-arrayed yeast colonies using a fluorimager as demonstrated by studies using the genome reporter matrix

30 (GRM) constructed by Acacia Biosciences. In the GRM, plasmid-borne *GFP* was placed under

the control of approximately 6,000 different yeast promoters and a high-density array of yeast colonies (wild-type cells) carrying the reporter constructs was monitored for genome-wide changes in gene expression in response to drug treatments.

In yet another embodiment of the invention, each of the yeast deletion mutants is constructed such that it is tagged with two unique 20mer oligonucleotide sequences. These "bar codes" allow for identification and analysis of specific deletion mutants within large populations. A microarray printed with probes for the bar codes that correspond to the approximately 5,000 viable deletion mutants can be used to follow synthetic lethality of particular strains following batch mating and sporulation experiments. Microarray-based synthetic lethal analysis with bar-coded mutants follows the same series of steps outlined in the pinning procedure for double-mutant construction. However, in this case, the yeast mutants are manipulated as a pooled population of cells and the growth of the cells is monitored as an array of bar codes. Thus, if a bar code is included into the components of the genetic array, manipulations of the cells can be carried out in batch format for array analysis via the bar codes. Details of the bar-code methodology are described in Example 12.

The major advantage of the microarray approach to synthetic lethal analysis stems from its experimental simplicity. In the microarray approach, the approximately 5,000 viable deletion mutants are manipulated as a single pooled population, which eliminates the need for high-density arrays of mutants and the volumes of media associated with manipulations of these arrays. Thus, with access to bar-coded microarrays and the pooled mutants, individual labs should be able to carry out synthetic lethal screens rapidly. Overall, the parallel development of both the systematic and microarray-based approaches to synthetic lethal screening will allow for exploitation of the strengths of each strategy.

Described above are a variety of methods and composition for comprehensive double-mutant construction in yeast that relies upon genetic manipulation to introduce a marked mutation or plasmid into an ordered array of yeast mutants. In another embodiment, mutations and plasmids can be introduced into an input array via standard transformation procedures, e.g. lithium acetate or electroporation for the transformation of yeast cells. In this case, the resultant transformants would form the output array.

Defined genetic alterations can be combined using genetic manipulations or strain transformation protocols. In one embodiment of the invention, large-scale double-mutant combinations are constructed using a specialized starting strain and an automated pinning method for manipulation of high density input arrays of defined starting yeast mutants. The

5   manipulations to be performed can be performed by a robot. Such robotic manipulations are described in detail in the examples, including Example 2. The development of robotic methods for manipulation of the budding yeast genome-wide deletion set will set the stage for exciting uses of the present invention. The high-density output arrays of multiple yeast strains of the present invention can be used in a variety of ways to analyze genetic interactions on a large-

10   scale, high throughput basis. A description of some of these uses follows.

Synthetic lethal analysis of high density output arrays assigning gene function

Mutations within many different yeast genes will lead to multiple synthetic lethal/fitness defects, generating a synthetic lethal profile for a given mutant. Cluster analysis of a set of

15   synthetic lethal profiles should identify mutant alleles that result in similar compromised states and therefore perturb similar functions within the cell (Fig. 3 and Fig. 4). Thus, large-scale synthetic lethal/fitness analysis with yeast genetic arrays will provide a method of determining gene function. Mutations in nonessential genes are most easily analyzed; however, conditional alleles of essential genes, i.e. those genes required for cell growth, e.g. temperature sensitive

20   alleles or those placed under the control of a regulated promoter (e.g. the Tet-regulated promoter), can be analyzed for synthetic effects following introduction into a genetic array. In the case of temperature sensitive alleles, the essential genes would be tested for synthetic lethal/fitness defects at a temperature below the nonpermissive/lethal temperature. For an essential gene under the control of a regulated promoter, synthetic lethal/fitness defects would be

25   tested at an intermediate gene expression level, i.e. at a level that compromises fitness but does not prevent cell growth.

30

Synthetic Lethal Analysis of High Density Output Arrays for Drug Discovery And Potential Cancer Therapeutics

The Seattle Project at the Fred Hutchison Cancer Research Center is founded on the concept that synthetic lethal analysis in model organisms provides a means to identify potential drug targets for cancer therapy. In this view, a null mutation represents a model for an ideal drug because in many cases it should mimic the effect of a highly specific inhibitor of the gene product. The identification of mutations that lead to a phenotype resembling a desired therapeutic outcome should thus also identify promising drug targets. Because the genes that control fundamental biological functions are often conserved, yeast and other model eukaryotes with sophisticated genetic methodology provide powerful systems for the identification of drug targets.

The rational identification of drugs for the treatment of particular forms of cancer or other diseases will likely stem from a knowledge of the mutations harbored by particular types of abnormal cells. In the context of anticancer drug therapeutics, the synthetic lethal analysis of a conserved yeast gene whose human homolog is often inactivated in tumors would identify potential targets for drug-induced inhibition. In this view, synthetic lethal analysis identifies genes whose inactivation results in cell death only within the context of another specific mutation and thus provides a potential program for killing cells within a diseased context.

Synthetic Lethal Analysis of High Density Output Arrays to Generate Cocktail Therapies

If synthetic lethal/fitness analysis identifies novel gene deletion mutations that show a synthetic effect in combination with a known drug or mutant allele of a known drug target, then the identified genes may represent a new target for a drug that will enhance the effectiveness of the known drug. For example, if we identify gene deletion mutants that are hypersensitive to the growth inhibitory effects associated with Cisplatin, a cancer therapeutic agent, then inhibitors of the identified genes would represent potential targets for drugs that would be used in combination with Cisplatin to kill cancer cells. In another example, if we identify gene deletion mutants that were hypersensitive to an antifungal drug, then the inhibitors of the identified genes

would represent potential targets for drugs that would used in combination with the antifungal

drug to kill a fungal pathogen. This procedure could also be used with any genetic manipulation

either defined above or elsewhere.

5

**Synthetic Lethal Analysis of High Density Input Arrays Screened
Against Small Molecules to Analyze Small Molecule-Target Interaction**

Comprehensive synthetic lethal analysis should provide us with a key for deciphering the

10   interactions between small molecules and biological targets in yeast. In this scheme, the

approximately 5,000 viable yeast disruption mutants are screened against small molecules of

interest for synthetic drug sensitivity. If the sensitivity to a particular molecule resulted from the

inactivation of a specific target then the synthetic drug sensitivity should mirror the synthetic

lethal profile of the target gene. Thus, a comprehensive synthetic lethal profile provides a key for

15   linking small molecules to biological targets in a whole cell growth assay.

The results of the comprehensive synthetic lethal analysis in yeast provide a whole cell

screen for inhibitors of specific target molecules. For example, to search for inhibitors of a the

PAK-like kinase Ste20p, we would simply screen for small molecules that specifically kill the

panel of yeast mutants carrying deletion mutations that are synthetically lethal when combined

20   with a *ste20Δ* mutation. The specificity of the screen will depend upon the specificity of the

synthetic lethal profile associated with the *ste20Δ* mutation. Given that each mutation will be

tested for synthetic lethality in approximately 5,000 different contexts, it is anticipated that even

genes within the same pathway may show a distinct synthetic lethal profile.

25   **Suppressor Analysis of a Conditional Lethal Situation**

Genetic arrays can be used to identify mutations that function as suppressors of lethality.

For example, consider a mutation in a gene whose product functions within a DNA damage

check point signal transduction pathway. The combination of a DNA damage check point

mutant and a DNA damaging agent leads to lethality and we can use a genetic array to screen for

30   gene deletion mutations that suppress the conditional lethal situation. As another example,

consider a gene that leads to lethality when overexpressed; in this case, we can use a genetic array to screen for mutations that are either hypersensitive or resistant to overexpression of the detrimental gene.

5   **Genetic Mapping and Backcrossing the
    Yeast Deletion Mutations into Another Genetic Background**

Use of the synthetic genetic arrays of the present invention should also allow for backcrossing the entire set of deletion mutations into another genetic background to analyze

10   traits specific to that background. Examples of genetic backgrounds which can be analyzed include the Σ1278 background that is competent for filamentous growth and the SK1 background that is hyperactive for sporulation.

Because haploid yeast double-mutants within the output array are formed by meiotic recombination, the analysis of strains within the output array can be used to map gain of function

15   phenotypes, even those that are multigenic traits. In this case, the gene(s) associated with the gain of function phenotype would fail to form double mutants efficiently with genetically linked gene deletions, resulting in a series of output strains that fail to inherit the gain of function phenotype.

20   **High Density Peptide Aptamer Mammalian Cell Microarrays**

The synthetic genetic array analysis of the present invention can be extended from yeast cells to mammalian cells by using an array of transfection constructs that lead to the expression of peptide or nucleic acid aptamers or other genetic alterations. In the case of peptide aptamer expression, the peptide aptamer expression plasmids are first suspended in gelatin solution and

25   arrayed on glass slides using a robotic microarrayer. Mammalian cells are then cultured on the glass slides containing the peptide aptamer expression plasmids. Cells growing in the vicinity of the gelatin spots uptake the peptide aptamer expression plasmids creating spots of localized transfection within a lawn of nontransfected cells. Once the peptide aptamer expression plasmids are incorporated into cells they can function as dominant agents, dominant agents being agents

30   which perturb the function of the cell in any way.

In one example, an input starting mammalian cell line might carry a set of genes that allow for the formation and detection of a two-hybrid interaction and the input array might carry a set of peptide aptamer expression plasmids. In this case, the output array would consist of mammalian cells transfected with the aptamers that may perturb the two-hybrid interaction. The

5   cell microarrays can be designed such that the positions of individual aptamers in the yeast array are cross-correlated to the positions of the same aptamers in the cell microarray. This correlation will allow aptamers that have observable phenotypes in the yeast array, much as the disruption of a mammalian protein interaction, to be directly assessed in the cell microarray.

As described above, there are numerous uses for the high density output arrays of

10   multiple yeast strains of the present invention, including but not limited to synthetic lethal analysis of high density output arrays to assign gene function, synthetic lethal analysis of high density output arrays for drug discovery and potential cancer therapeutics, synthetic lethal analysis of high density output arrays to generate cocktail therapies, synthetic lethal analysis of high density input arrays screened against small molecules to analyze small molecule-target

15   interaction, and suppressed analysis of a conditional lethal silation.

This invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

20   **EXAMPLES**

Example 1 - Creation of Double Mutant Output Array

An example of a starting strain that could be used in this embodiment is the *Saccharomyces cerevesiae* strain termed Y2454. The Y2454 strain is characterized by being a

25   *MAT*□ mating type with ura3, leu2, his3, and lys2 mutations, and a HIS3 gene linked to an MFA1 promoter. The ura3, leu2, his3, and lys2 mutations require the strain to be grown in supplemented media to survive. They also carry a can1 null allele which confers canavinine resistance to the cells. A mutant gene, for example, one of the approximately 5,000 non-lethal

mutations found in *Saccharomyces cerevesiae*, is introduced into this strain. The deleted gene is being replaced by a *NAT* gene which confers noureseothricin resistance to these cells.

This strain can be crossed with a starting array of yeast strains of the *MAT***a** mating type. The strains in this starting array contain ura3, leu2, his3, and met15 knockouts, so that they can only survive on supplemented media. These cells can also contain a mutation of one of the approximately 5,000 non-lethal gene deletions known in *Saccharomyces cerevesiae*. The deleted gene is replaced with an operably linked *KAN* gene, which gives the yeast cells resistance to kanamycin derivatives like Geneticin. These starting array strains carry a wild-type CAN1 locus, which makes them sensitive to canavinine.

A double mutant haploid cell could then be developed by the following steps:

Step 1. Construction of a Y2454-derivative that carries a *nat*-marked mutant allele, e.g. *bni1Δ::nat* where the given gene is deleted and replaced with *nat,* which results in nourseothricin-resistance, for genome-wide synthetic lethal analysis.

Step 2. Mating of the *MAT*α Y2454-derivative to the array of *MAT***a** *xxxΔ::kan* deletion mutants on rich medium to facilitate zygote formation.

Step 3. Transfer zygotes to medium containing geneticin and nourseothricin to select for growth of *MAT***a**/α diploid cells.

Step 4. Transfer *MAT***a**/α diploid cells to sporulation medium to induce spore formation.

Step 5. Transfer spores to synthetic medium lacking histidine and containing canavanine to select for growth *MAT***a** haploid spore-progeny.

Step 6. Transfer *MAT*a haploid spore-progeny to medium containing geneticin and nourseothricin to score for growth and viability of *MAT*a *bni1*Δ*::nat xxx*Δ*::kan* double-mutants.

In step 2, yeast cells are arrayed on rich medium to allow efficient mating. In step 3, the mating reactions are arrayed onto medium containing geneticin and nourseothricin, which allows for selected growth of diploid cells. In step 4, the diploid cells are transferred to medium that is low in nitrogen and carbon and induces sporulation. In step 5, the spores are transferred to germination medium that selects for growth of the haploid *MAT*a cells (this step is described in detail below). In step 6, double mutant strains are selected for growth the two mutations are scored as synthetic lethal/fitness defect if the *MAT*a haploid double-mutants form a colony that is smaller than that associated with either of the single mutants. The mutations predicted to be synthetically lethal can be analyzed in more detail through tetrad analysis of the heterozygous diploid cells created in Step 2.

Example 2 - Use of Robotics to Generate Output Array

Following construction of a *natR*-marked mutant for synthetic lethal analysis, simple replica plating or pinning manipulations will enable us to complete steps 2-6 of Example 1. These steps can be carried out through the use of a robotic colony arrayer, as described below. The Colony Arrayer used in this Example, the Virtek Vision CPCA was designed by our labs in conjunction with Virtek Vision. The CPCA is based upon a system used for genome-wide two-hybrid arrays, since the manipulations required for automated two-hybrid screens are very similar to those required for automated double-mutant construction and synthetic lethal screens.

For a rapid genome-wide two-hybrid screening procedure, we have created a robotic colony array which includes replica-plate pinhead that transfers 768 individual colonies from one standard microtiter-sized agar-slab plate to another in a single move. This cell density allows efficient mating, on the order of hundreds of zygotes formed per spot pinned, a frequency that will easily suffice for genome-wide synthetic lethal screens.

The general specifications of a colony arrayer are as follows:

1. 16 input and 16 output plates;

2. Replicating Pinning Head with 96, 384, or 768 pins, for high speed transfer of the colonies of different array density;

3. A gripper for handling the plates, and if desired for removing/replacing the covers for full automation;

4. Wash/Dry station for cleaning the pins between runs and includes the 5 stages of water, ethanol or bleach, sonicator (water), ethanol, and air-drying;

5. Controlled environment enclosure, with HEPA filter, humidifier, positive pressure and internal UV lamps to ensure full sterilization of the environment before arraying process.

Example 3 - Recovery of Haploid Spore Progeny

The recovery of haploid spore progeny is mentioned in step 5 in Example 1, and is described in greater detail below. The *MATα* starting strain described in Example 1, Y2454, carries two selectable markers, *can1Δ0* and *MFA1pr-HIS3*, both of which permit efficient recovery of haploid spore progeny.

i) *MFA1pr-HIS3*

MFA1 encodes the a-factor precursor, which is expressed constitutively in *MATa* cells. The *MFA1* promoter, *MFA1pr*, is repressed in *MATα* and *MATa /α* cells. The *MFA1pr-HIS3* reporter was constructed by replacing the *MFA1* ORF (open reading frame) with the *HIS3* ORF such that *MFA1pr* drives *HIS3* expression. The *MATα* starting strain, Y2454, fails to grow on synthetic medium lacking histidine because the *MFA1pr-HIS3* reporter is repressed in *MATα* cells. The cells constructed in step 2 of Example 1 will also fail to grow on synthetic medium lacking histidine because the *MFA1pr-HIS3* reporter is repressed in *MAT* a/α cells. Following sporulation of the diploid cells in Step 4 of Example 1, the *MFA1pr-HIS3* reporter selects for growth of *MATa* haploid progeny. Twenty-five per cent of the haploid progeny generated by sporulation of the diploid cells will be *MATa MFA1pr-HIS3*. Other a-specific reporters can be constructed using promoters from different a-specific genes (e.g. *MFA2, ASG7, STE2*) or

different reporters (e.g. *URA3, LEU2,* or heterologous genes conferring resistance to antibiotics or other chemicals)

ii) *can1Δ0*

5   The *CAN1* gene encodes an arginine permease. The Y2454 starting strain has been engineered to carry a recessive *can1Δ0* null allele, which renders the cells resistant to canavanine, a toxic arginine analog that is transported by the *CAN1* gene product. The knock-out strains constructed by the deletion consortium are canavanine-sensitive because they carry a wild-type *CAN1* locus. The *MATα* starting strain, Y2454, carries *can1Δ0* rendering it

10   canavanine-resistant. The *MATa/α can1Δ0/CAN1* diploids isolated in step 3 of Example 1 will be canavanine-sensitive. Following sporulation of the diploid cells in step 4 of Example 1, the *can1Δ0* allele allows for selection of canavanine-resistant haploid progeny. Fifty percent the haploid spore progeny will be canavanine-resistant. Other recessive drug resistant genes, such as cyh2 mutations which leads to cycloheximide resistance, can also be used.

Efficient Selection for Haploid Spore Progeny

       Both the *can1Δ0* and the *MFA1pr-HIS3* reporter allow selection for haploid spore progeny in Step 5 of the pinning procedure described in Example 1. The selection provided by the *MFA1pr-HIS3* reporter enables the specific isolation of *MATa* cells. It is important to isolate

20   spore progeny of a single mating type when ultimately scoring for the presence or absence of two marked mutant alleles; otherwise progeny of opposite mating type may conjugate to generate diploid cells heterozygous for each mutation, which would appear, perhaps falsely, as a viable double-mutant.

       Initially, we tested if the *MFA1pr-HIS3* reporter would suffice for isolation of *MATa*

25   haploid spore progeny in step 5. These tests revealed that, prior to incubation on sporulation medium, a fraction of the *MATa/α MFA1-HIS3/MFA1* cells became competent for growth on medium lacking histidine. This process appears to involve mitotic recombination between the centromere and the *MAT* locus on chromosome III, creating *MATa/a MFA1-HIS3* cells, which

express *MFA1-HIS3* and grow on medium lacking histidine. In a synthetic lethal screen, these *MATa/a* diploid cells would be heterozygous for the *NAT*-marked and the *KAN*-marked alleles and appear as viable double-mutant haploid cells. To overcome this problem, we introduced a *can1Δ0* mutation into our starting strain; *can1* mutant alleles have been used extensively to select for canavanine-resistant haploids in random spore analysis. Analogously, the *MATa/α can1Δ0/CAN1 MFA1pr-HIS3/MFA1* diploids, constructed in steps 2 and 3, can become canavanine-resistant as diploid cells through mitotic recombination involving the *can1Δ0* locus, creating *MATa/α can1Δ0/can1Δ0 MFA1pr-HIS3/MFA1* cells. Importantly, prior to incubation on sporulation medium, we do not observe the formation of cells that are both canavanine-resistant and competent for growth on medium lacking histidine. Thus, the frequency of these two distinct mitotic recombination events is below the level of detection and we will not observe *MATa/a can1Δ0/can1Δ0 MFA1pr-HIS3/MFA1* cells as background during a synthetic lethal screen.

Example 4 - Genetic Exceptions

4A. xxxΔ::kanR Deletion Mutations Linked to *MFA1pr-HIS3* or *can1Δ*

While the *MFA1pr-HIS3* and the *can1Δ* alleles are essential for synthetic lethal analysis via a pinning procedure, they present a position-based problem. The *xxxΔ::KANR* deletion mutations that are linked tightly to these alleles will be paired into the genome of the haploid spores at reduced frequency and may lead to a false synthetic lethal score. However, because the position of every ORF is defined precisely, the problematic double-mutant combinations are predictable. For mutations in the vicinity of *MFA1pr-HIS3* on chromosome X, we will employ an *MFA2pr-HIS3* starting strain. The *MFA2* gene encodes a second copy of the a-factor structural gene. Like *MFA1pr*, *MFA2pr* leads to a-specific gene expression and will facilitate selection for *MATa* spores. Because 1 cM in *S. cerevisiae* is roughly equivalent to 1.5 Kb of DNA sequence and the average *S. cerevisiae* gene is approximately 2 Kb, we anticipate that 10-30 genes on either side of the *MFA1* locus will have to be mated to a starting strain containing *MFA2pr-HIS3*. Alternatively the *MFA1pr-HIS3* could be moved to another position in the genome. The *can1Δ0* allele presents a slightly different problem because it cannot be moved like

the *MATa*-specific reporter. To solve this problem, we will directly introduce the *can1*Δ allele into approximately 10-30 of the *xxx*Δ::*kanR* deletion mutants on either side of the *CAN1* locus. Alternatively, we could employ another recessive drug resistant marker, e.g. *cyh2*.

5 **4B. Genetic Interactions with Selection Genes**

Some *xxx*Δ::*kanR* deletion mutations may cause synthetic lethality in synthetic lethal starting strains carrying the *MFA1pr-HIS3* and *can1*Δ alleles. Because *MFA1* is a specialized gene devoted to conjugation, it is unlikely that *MFA1pr-HIS3* will be associated with any synthetic lethal interactions unless localized alterations of the genome affect the function of

10 neighboring genes. Because the *CAN1* gene product facilitates arginine uptake, the *can1*Δ deletion mutation will be synthetically lethal with any gene that prevents arginine biosynthesis. All the genes required for arginine biosynthesis will be defined/confirmed simply by creating all the double mutants with the wild-type version of the starting strain.

15 **4C. Defects in Mating and Spore Formation**

Some *xxx*Δ::*KANR* deletion mutations will be defective for one of the cellular functions required for double mutant construction or spore formation. For example, mating defective mutants (e.g. *ste4*Δ) will fail to form diploids in Step 2 of the pinning procedure. A genome-wide synthetic lethal screen will also identify all the genes required for sporulation in Step 4.

20 Obviously, diploids that are homozygous for certain mutations will lead to a sporulation defect; however, other genes may prove haploinsufficient for sporulation or two mutations may exhibit nonallelic-noncomplementation, giving rise to a sporulation defect. To distinguish the mutants defective for these functions, analysis of the growth of *MATa MFA1pr-HIS3 can1*Δ0 cells in Step 5 will be important.

25

Example 5 - Comprehensive Synthetic Lethal Screen in Yeast

A large-scale comprehensive synthetic lethal analysis can be performed either by constructing approximately 5,000 gene deletions in the synthetic lethal starting strain, Y2454, or

by establishing an automated method to transfer the knockout alleles constructed by the deletion consortium into the Y2454 starting strain. In this example, we utilize an automated introduction of the deletion consortium knockout alleles into a synthetic lethal starting strain. First we switch each *kanR*-marked allele to a *natR*-marked allele and then select for the presence of *MFA1pr-*

5   *HIS3* in the *MAT*α synthetic lethal starting strain following a genetic cross and a series of pinning steps.

Switching the *kanR*-marked allele to a *natR*-marked allele is achieved easily through transformation of *xxxΔ::KAN* strains with a *kanR-natR* switcher-cassette, in which the *natR* gene has been engineered so that it is flanked by sequences within the original *kanR* disruption cassette, and subsequent screening of the *nat*-resistance transformants for kanamycin sensitivity.

10   Strain growth and transformation will be carried out using a 96 well format; therefore, approximately 52 rounds of transformation will allow us to switch the panel of approximately 5000 viable *MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 xxxΔ::kan* strains to *MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 xxxΔ::nat* strains.

The next challenge is to move the approximately 5,000 *xxxΔ::nat* alleles from the *MAT***a**

15   *ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 xxxΔ::nat* cells into the strain that carries the haploid selection alleles, *MFA1pr-HIS3* and *can1Δ0*. Of the alleles specific for the *MAT*α strain, *lys2Δ0* and *can1Δ0* are associated with drug-mediated selections; however, we must construct a haploid selection marker that is functionally equivalent to *MFA1pr-HIS3* and selects specifically for

20   growth of *MAT*α cells.

Construction of two tightly linked reporters, one that selects for *MAT***a** cells and another that selects for *MAT*α cells, provides a solution to this problem. A dual reporter, *MFA1pr-HIS3::MFα1pr-LEU2*, will be created simply by integrating *MFα1pr-LEU2* just downstream of *MFA1pr-HIS3*. As described above, the *MFA1pr-HIS3* reporter provides a selection for haploid

25   *MAT***a** cells on medium lacking histidine. *MFα1pr* controls the expression of the α-factor structural gene and is expressed only in *MAT*α cells. *MFα1pr-LEU2* provides a selection for *MAT*α cells on synthetic medium lacking leucine. Because *MFA1pr-HIS3* is tightly linked to *MFα1pr-LEU2* within the context of the dual reporter, the *MAT*α cells recovered on synthetic medium lacking leucine will also carry *MFA1pr-HIS3*. Several variations of this theme are

possible. For example, the *MFA1pr-HIS3* could be placed at *CAN1* locus, creating

can1Δ0::MFA1pr-HIS3; in this context, growth of *MATα MFα1pr-LEU2 can1Δ0::MFA1pr-HIS3*cells can be selected on synthetic medium containing canavanine and lacking leucine.

5 **Experimental Steps for a 5,000 X 5,000 Genome-wide Synthetic Lethal Screen**

Step 1. 96-well format transformation will be used to switch a panel of approximately 5000 viable *MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 xxxΔ::kanR* strains to *MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 xxxΔ::NAT* strains. Transformants that grow on medium containing nourseothricin will be screened for Geneticin sensitivity to confirm the switching event.

10

Step 2. The *MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 xxxΔ::natR* strains will be mated to *MATα ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 MFA1pr-HIS3::MFα1pr-URA3 can1Δ0* on rich medium. The resultant diploid cells will be selected for growth on synthetic medium lacking methionine and lysine. As mentioned above, this selection is not ideal because the *met15Δ0* allele does not completely eliminate growth on medium lacking methionine; however, because each strain will only require mating once, we will be able to follow the diploid selection carefully using relatively large patches of cells and double replica plating to selective medium.

Step 3. Sporulation of the resultant diploid cells and selection for *MATα ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 MFA1pr-HIS3::MFα1pr-URA3 can1Δ xxxΔ::LEU2* on medium that lacks uracil and leucine but contains α-aminoadipate and canavanine. α-aminoadipate selects for *lys2Δ0* mutant cells and canavanine selects for *can1Δ0* cells; 50% of the resultant haploids will be *met15Δ0*. The presence of the *met15* marker can be scored by successive replica-platings to medium lacking methionine.

25

Step 4. Each of the approximately 5000 *MATα ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 MFA1pr-HIS3::MFα1pr-URA3 can1Δ xxxΔ::LEU2* strains will each be mated to the array of *MATa*

*xxx*Δ::*KANR* deletion mutants and run through steps 2-6 of the pinning procedure outlined in Example 1 for systematic genome-wide synthetic lethal analysis.

Example 6 - Input Array of 5,000 Strains Crossed with Particular Mutation

In a large-scale application of the synthetic genetic analysis of the present invention, we screened a *bni1*Δ query mutation against an array of 4,644 different viable deletion strains. The array was propagated on a set of agar plates at a density of 384 strains per plate. For screening, the array was first pinned manually and subsequently adapted for high-throughput automation using robotics that we designed specifically for the manipulation of high density yeast arrays. To ensure reproducibility within a screen and to facilitate visual scoring, each gene deletion strain was arrayed in pairs, at 768 strains per plate. We scored 67 potential synthetic lethal/sick interactions, 51 (76%) of which were confirmed by tetrad analysis. To group the identified genes by functional classification, we assembled a list of their cellular roles defined by the Yeast Proteome Database . As shown in Table 1, these interactions were highly enriched for genes with roles in cell polarity (20%) cell wall maintenance (18%), and mitosis (16%). Pathways critical for the fitness of *bni1*Δ cells were revealed by multiple interactions with subsets of genes involved in bud emergence (*BEM1*, *BEM2*, and *BEM4*), chitin synthase III activity (*CHS3*, *SKT5*, *CHS5*, *CHS7*, and *BNI4*), MAP kinase pathway signaling (*BCK1* and *SLT2*), the cell cycle-dependent transition from apical to isotropic bud growth (*CLA4*, *ELM1*, *GIN4*, and *NAP1*), and the dynein/dynactin spindle orientation pathway (*DYN1*, *DYN2*, *PAC1*, *PAC11*, *ARP1*, *JNM1*, *NIP100*). Importantly, we identified 8 of the 10 previously known *bni1*Δ synthetic lethal/sick interactions, which include *BNR1*, *HOF1*, *CDC12*, *SLT2*, *BCK1*, *PKC1*,genes implicated in polarized morphogenesis and cell wall maintenance, and *ASE1*, *DYN1*, *ARP1*, *NIP100*, genes implicated in mitotic spindle function . Of those that were not identified, *CDC12* and *PKC1* were not contained in our deletion set, while cells lacking *HOF1* grow very slowly and are apparently beyond the sensitivity of the assay. In total, we discovered 42 novel synthetic genetic interactions for *bni1*Δ, including 10 genes of unclassified function.

Table 1 below lists a set of synthetic lethal/sick interactions observed for query mutations in *BNI1*, *ARC40*, *BBC1*, *NBP2*, *BIM1*, *RAD27*, and *SGS1*.

TABLE 1

BNI1	Cell Role
BEM1	Cell Polarity
BEM2	Cell Polarity
BEM4	Cell Polarity
BUD6[t]	Cell Polarity
SLA1[t]	Cell Polarity
CLA4	Cell Polarity
ELM1[t]	Cell Polarity
GIN4	Cell Polarity
NAP1[t]	Cell Polarity
SWE1[t]	Cell Polarity
BNR1	Cytokinesis
CYK3[t]	Cytokinesis
SHS1	Cytokinesis
BCK1	Cell Wall Maintenance
BNI4[t]	Cell Wall Maintenance
FAB1	Cell Wall Maintenance
CHS3	Cell Wall Maintenance
SKT5[t]	Cell Wall Maintenance
CHS5[t]	Cell Wall Maintenance
CHS7[t]	Cell Wall Maintenance
SLT2	Cell Wall Maintenance
SMI1[t]	Cell Wall Maintenance
ARP1	Mitosis
ASE1	Mitosis
DYN1	Mitosis
DYN2[t]	Mitosis
JNM1	Mitosis
NIP100	Mitosis
NUM1	Mitosis
PAC1	Mitosis
ATS1	Cell Structure
PAC11	Cell Structure
YKE2[t]	Cell Structure
PCL1[t]	Cell Cycle Control
DRS2	RNA Processing
SNC2	Vesicular Transport
VPS28	Vesicular Transport
YPT6[t]	Vesicular Transport
ELP2	Pol II Transcription
ELP3[t]	Pol II Transcription
BBC1[t]	Unknown
NBP2[t]	Unknown
TUS1[t]	Unknown
YBL051c[t]	Unknown
YBL062w[t]	Unknown
YDR149c	Unknown
YHR111w[t]	Unknown
YKR047w[t]	Unknown
YLR190w[t]	Unknown
YMR299c[t]	Unknown
YNL119w[t]	Unknown

BBC1	Cell Role
BEM1	Cell Polarity
BEM4	Cell Polarity
BNI1[t]	Cell Polarity
SLA1[t]	Cell Polarity
CAP1[t]	Cell Structure
CAP2[t]	Cell Structure
PAC10[t]	Cell Structure
GIM3	Cell Structure
GIM5	Cell Structure
SAC6	Cell Structure
CHS5[t]	Cell Wall Maintenance
RAS2	Signal Transduction
ELP2[t]	Pol II Transcription
ELP3[t]	Pol II Transcription
SDS3	Chromatin Structure
YLR235c	Unknown
YML095c-A	Unknown

ARC40	Cell Role
ARC18	Cell Polarity
BEM1	Cell Polarity
BEM2	Cell Polarity
CLA4	Cell Polarity
MYO5[t]	Cell Polarity
PEA2[t]	Cell Polarity
VRP1	Cell Polarity
BCK1	Cell Wall Maintenance
BNI4[t]	Cell Wall Maintenance
CHS3	Cell Wall Maintenance
SKT5	Cell Wall Maintenance
CHS5	Cell Wall Maintenance
CHS6	Cell Wall Maintenance
CHS7	Cell Wall Maintenance
HOC1	Cell Wall Maintenance
KRE1	Cell Wall Maintenance
SLT2	Cell Wall Maintenance
SPF1[t]	Cell Wall Maintenance
YER083c[t]	Cell Wall Maintenance
YKE2	Cell Structure
GIM3	Cell Structure
GIM4[t]	Cell Structure
CCT3[t]	Cell Structure
SAC6	Cell Structure
GLO3	Vesicular Transport
SAP155[t]	Cell Cycle Control
SEC66	Protein Modification
ILM1	Energy Generation
MNN11	Protein Modification
STE24[t]	Protein Modification
CIK1[t]	Meiosis
RIM101[t]	Meiosis
RUD3	Vesicular Transport
SEC22	Vesicular Transport
TFP3	Small Molecule Transport
CPR7[t]	Protein Folding
SHE4	Differentiation
SUM1[t]	Pol II Transcription
YBL062w	Unknown
YLR111w[t]	Unknown

ARP2	Cell Role
BEM1[t]	Cell Polarity
BEM2[t]	Cell Polarity
CLA4[t]	Cell Polarity
PEA2[t]	Cell Polarity
PRK1[t]	Cell Polarity
RGD1[t]	Cell Polarity
RVS161	Cell Polarity
RVS167[t]	Cell Polarity
VRP1[t]	Cell Polarity
BCK1	Cell Wall Maintenance
BNI4	Cell Wall Maintenance
CHS3	Cell Wall Maintenance
SKT5	Cell Wall Maintenance
CHS5[t]	Cell Wall Maintenance
CHS6[t]	Cell Wall Maintenance
CHS7[t]	Cell Wall Maintenance
HOC1	Cell Wall Maintenance
KRE1	Cell Wall Maintenance
SLT2	Cell Wall Maintenance
SPF1[t]	Cell Wall Maintenance
YER083c	Cell Wall Maintenance
YKE2	Cell Structure
PAC10	Cell Structure
GIM3	Cell Structure
GIM4	Cell Structure
SAC6	Cell Structure
SAC7[t]	Cell Structure
ILM1	Energy Generation
SAP155[t]	Cell Cycle Control
SEC66	Protein Modification
MNN11	Protein Modification
STE24[t]	Protein Modification
BTS1	Protein Modification
RUD3	Vesicular Transport
CPR7	Protein Folding
SHE4[t]	Differentiation
SUM1	Pol II Transcription
SRO9	Protein Synthesis
UTH1[t]	Aging
DEP1	Lipid Metabolism
YBL062w	Unknown
YDR018c	Unknown
YGL250w[t]	Unknown
YLR111w[t]	Unknown

BIM1	Cell Role
ARP1	Mitosis
ASE1	Mitosis
BIK1	Mitosis
CHL4	Mitosis
DYN1	Mitosis
JNM1	Mitosis
KIP3	Mitosis
NUM1	Mitosis
PAC1	Mitosis
SLK19	Mitosis
BFA1	Mitosis
BUB1	Mitosis
BUB2	Mitosis
BUB3	Mitosis
MAD1	Mitosis
MAD2	Mitosis
MAD3	Mitosis
CSM3	Meiosis
MCK1	Meiosis
CTF8	Chromatin Structure
CTF19	Chromatin Structure
DCC1	Chromatin Structure
IML3	Chromatin Structure
MCM21	Chromatin Structure
MCM22	Chromatin Structure
PHO23	Chromatin Structure
SAP30	Chromatin Structure
BEM1	Cell Polarity
ARP6[t]	Cell Structure
GIM3	Cell Structure
GIM4	Cell Structure
GIM5	Cell Structure
PAC11	Cell Structure
FAB1	Cell Wall Maintenance
SMI1	Cell Wall Maintenance
ELP2	Pol II Transcription
INP52	Vesicular Transport
RAD54	DNA Repair
PPZ1	Signal Transduction
KEM1	RNA Processing
AOR1	Unknown
IES2[t]	Unknown
MRC1	Unknown
RTT103[t]	Unknown
VID22	Unknown
YTA7	Unknown
YBR095c	Unknown
YDR149c	Unknown
YGL211w[t]	Unknown
YGL217c	Unknown
YNL170w	Unknown
YLR381w	Unknown
YLR386w	Unknown
YML095c-A	Unknown
YPL017c	Unknown

NBP2	Cell Role
BIM1	Mitosis
CIN2[t]	Mitosis
KAR9	Mitosis
KIP3	Mitosis
PAC10	Cell Structure
GIM5	Cell Structure
CAP1[t]	Cell Structure
CAP2[t]	Cell Structure
BNI1[t]	Cell Polarity
FAB1	Cell Wall Maintenance
SMI1[t]	Cell Wall Maintenance
VAM7[t]	Vesicular Transport
VPS29[t]	Vesicular Transport
RPL16A[t]	Protein Synthesis
RPS18B[t]	Protein Synthesis
RPS23A[t]	Protein Synthesis
CPR6[t]	Protein Folding
FPR1[t]	Protein Folding
CLB4[t]	Cell Cycle Control
REM50[t]	DNA Repair
RTG2[t]	Carbohydrate Metabolism
RTG3[t]	Carbohydrate Metabolism
MON1[t]	Unknown
YDL063c[t]	Unknown
YGL211w[t]	Unknown
YGL217c	Unknown
YML095c-A	Unknown

SGS1	Cell Role
ASF1[t]	DNA Repair
HPR5	DNA Repair
POL32	DNA Repair
RAD27	DNA Repair
RAD50	DNA Repair
SAE2	DNA Repair
SLX1	DNA Repair
MMS4	DNA Repair
MUS81	DNA Repair
SLX4	DNA Repair
WSS1	DNA Repair
RNR1	DNA Synthesis
RRM3	DNA Synthesis
YNL218w[t]	DNA Synthesis
CSM3[t]	Meiosis
ESC2[t]	Chromatin Structure
ESC4[t]	Chromatin Structure
TOP1[t]	Chromatin Structure
SWE1[t]	Cell Cycle Control
PUB1[t]	RNA Processing
RPL24A[t]	Protein Synthesis
SIS2[t]	Cell Stress
SOD1	Cell Stress
YBR094w	Unknown

RAD27	Cell Role
DDC1[t]	DNA Repair
EXO1[t]	DNA Repair
HPR5	DNA Repair
MRE11	DNA Repair
MMS4[t]	DNA Repair
MUS81[t]	DNA Repair
RAD9	DNA Repair
RAD17[t]	DNA Repair
RAD24[t]	DNA Repair
RAD50	DNA Repair
RAD51	DNA Repair
RAD52	DNA Repair
RAD54	DNA Repair
RAD55	DNA Repair
RAD57	DNA Repair
SAE2	DNA Repair
XRS2	DNA Repair
CTF4[t]	DNA Synthesis
CAC2[t]	Chromatin Structure
ESC2[t]	Chromatin Structure
HST1[t]	Chromatin Structure
HST3[t]	Chromatin Structure
HPC2[t]	Pol II Transcription
CSM3[t]	Meiosis
DOC1	Cell Cycle Control
RPL27A	Protein Synthesis
RPS30B[t]	Protein Synthesis
YDJ1	Protein Translocation
LYS7	Cell Stress
SIS2[t]	Cell Stress
SOD1	Cell Stress
FYV11	Unknown
YLR352w[t]	Unknown
YNL171c	Unknown
YPR116w	Unknown

Example 7 - Demonstration that
Pinning Procedure Works for Synthetic Lethal Analysis

The present example demonstrates that the replica pinning procedure of the present

5   invention can be used for synthetic lethal analysis. A deletion of the *BNI1* gene, *bni1*Δ, was

selected as the query mutation. A test-array of gene deletion mutants was assembled that

included *bnr1*Δ, which is synthetically lethal with *bni1*Δ. *BNI1* and *BNR1* both encode members

of the formin family, proteins that appear to control actin polymerization in response to signaling

by Rho-type GTPases. Growing yeast cells contain two major filamentous actin structures,

10   cortical actin patches, which polarize to the cortex of the growing bud and act as sites of

endocytosis, and actin cables, which align along the mother bud axis and act as tracks for myosin

motors that coordinate polarized cell growth and spindle orientation. Bni1 and Bnr1 are required

for the formation of actin cables. *bni1*Δ mutants show defects in polarized cell growth and

spindle orientation, whereas *bnr1*Δ mutants display no obvious phenotype, indicating that

15   Bni1 functions as the predominant formin in yeast cells. The array contained 96 strains, each of

which was included in quadruplicate and positioned next to each other in a square pattern,

resulting in a matrix with 384 elements. *bnr1*Δ was included at two positions and enriched the

array for mutations in other genes with roles in actin assembly and cell polarity.

The array of *bni1*Δ double-mutants resulting from the final pinning and the corresponding

20   wild-type control are shown in Fig. 2A. As anticipated, the cells at the *bnr1*Δ positions failed to

grow, forming a residual colony with a reduced size relative to the control. Because the resultant

double-mutants are created by meiotic recombination, gene deletions that are genetically linked

to the query mutation form double mutants at a reduced frequency. Moreover, when the query

mutation is actually identical to one of the gene deletions within the array, double mutants can

25   not form. Thus, we also anticipated that the cells at the *bni1*Δ position would fail to grow under

double-mutant selection. Novel synthetic genetic interactions were observed with gene deletion

mutations of *CLA4* and *BUD6* (*AIP3*). Cla4 is a PAK-like kinase involved in actin patch

assembly and the cell cycle-dependent transition from apical to isotropic bud growth; Bud6

forms a complex with Bni1 and actin to control actin cable assembly and cell polarity. Tetrad

30   analysis confirmed that both the *bni1*Δ *bnr1*Δ and *bni1*Δ *cla4*Δ double-mutants were inviable and

that the *bni1Δ bud6Δ* double-mutant was associated with a slower growth rate or "synthetic sick" phenotype, reflecting reduced fitness of the double-mutant relative to the respective single mutants (Fig. 2B). This example thus demonstrates that the replica pinning procedure can identify genetic interactions corresponding to the spectrum of fitness defects from synthetic sick

5   to synthetic lethal phenotypes.

Example 8 - Input Array of 5,000 Strains
Crossed with Uncharacterized Query Mutation

10   If synthetic lethal/sick interactions identify functionally related genes, then some of the uncharacterized genes identified in the *bni1Δ* screen should also participate in cortical actin assembly and cell polarity. To test this possibility, we conducted synthetic lethal/sick screen for a previously uncharacterized gene, *BBC1*. The *bbc1Δ* deletion mutant shows no obvious phenotype and the Bbc1 amino acid sequence is not indicative of a cellular role; however, Bbc1

15   contains an SH3 protein-protein interaction domain, suggesting that Bbc1 may function as part of a complex. We scored 16 potential synthetic lethal/fitness interactions for *bbc1Δ*, the majority of which have YPD-classified cell polarity or cell structure (cytoskeletal) roles (Table 1). In particular, *bbc1Δ* showed interactions with several genes whose products control actin polymerization and localize to cortical actin patches (*CAP1*, *CAP2*, *SAC6*, and *SLA1*), suggesting

20   *BBC1* may be involved in the assembly actin patches or their dependent processes. The results of additional experiments demonstrated that Bbc1 localized predominantly to cortical actin patches and that its SH3 domain binds directly to Las17 (Bee1), a member of the WASP (Wiskott-Aldrich syndrome protein) family proteins that controls the assembly of cortical actin patches through regulation of the Arp2/3 actin nucleation complex .

25

Example 9 - Input Array of 5,000 Strains Crossed with Helicase Mutation

To explore the potential for the synthetic genetic analysis of the present invention to identify interactions for genes with roles distinct from cytoskeletal organization and cell polarity, we undertook screens with two non-essential genes that function in DNA damage and repair

pathways. *SGS1* encodes the yeast homolog of the human Werner's Syndrome protein, WRN, a member of the RecQ family of DNA helicases, while *RAD27* encodes an enzyme that processes Okazaki fragments during DNA synthesis and repair. The phenotype of yeast cells deleted for the *SGS1* gene mirrors the chromosomal instabilities and premature aging associated with

5 Werner's syndrome. *SGS1* is known to show a synthetic lethal/sick relationship with *YNL218W*, which encodes the yeast homolog of human Werner helicase interacting protein, and seven other genes (*SLX1, MMS4, SLX3, SLX4, HEX3, HRP5,* and *SLX8*), which are thought to mediate the resolution of recombination intermediates generated in the absence of *SGS1*. In total, we scored 24 potential synthetic lethal/fitness interactions for *SGS1*, the majority of which were associated

10 with cellular roles in DNA synthesis and repair (Table 1). We identified 6 of the previously known synthetic lethal/sick interactions; the 2 that were missed (*HEX3, SLX8*) had severe growth defects that precluded propagation through the multiple pinning steps. Several novel *SGS1* synthetic lethal/sick interactions are of considerable interest: *RRM3*, a gene that encodes a closely related helicase involved in rDNA replication; *WSS1*, a gene identified as a high dosage

15 suppressor of *SMT3*, which codes a conserved ubiquitin-related protein that interacts with *HEX3* in the two-hybrid system ; *YBR094W*, a highly conserved gene of unknown function with an NH2-terminal SurE domain and COOH-terminal tubulin-tyrosine ligase-like domain; and *ESC4*, which codes for an uncharacterized gene with 3 BRCT domains, peptide recognition modules that appear to be found exclusively in proteins involved in DNA synthesis and repair.

20

Example 10 - Network of Synthetic Lethal Analysis

Because many of the synthetic lethal/sick relationships appear to involve functionally related genes, the data set derived from large-scale synthetic genetic interactions should form a highly connected network in which genes are classified base upon their connectivities. To build

25 a network of interactions and further explore the potential of this methodology, we conducted additional synthetic lethal/sick screens with specific query mutations in the following genes:

(i) *bim1Δ*, a complete deletion of *BIM1*, whose product localizes to the tips of astral microtubules and controls the orientation of the mitotic spindle.

(ii) *nbp2Δ*, a complete deletion of *NBP2*, gene of uncharacterized function that showed a synthetic lethal/sick interaction with *BNI1*; the Nbp2 product contains an SH3 domain and shows a two-hybrid interaction with Nap1.

(iii) *arc40-1*, a temperature sensitive allele of *ARC40*, an essential gene that codes for a component of the Arp2/3 complex, a seven-member complex that functions to nucleate actin filaments, controlling the assembly, movement, and localization of cortical actin patches in yeast.

(iv) *arp2-2*, a temperature sensitive allele of *ARP2*, coding for one of the key actin-related proteins of the Arp2/3 complex.

(v) *rad27Δ*, a complete deletion of *RAD27*, which showed a synthetic lethal/sick interaction with *SGS1* and whose product functions as a nuclease that processes Okazaki fragments during DNA synthesis.

To plot the resultant synthetic lethal/sick interactions as a network, the data set was first imported into the Biomolecular Interaction Network Database (BIND), then formatted with BIND tools and exported to the Pajek package, a program originally designed for the analysis of social networks. The genetic interaction network shown in Fig. 3 contains 205 genes, represented as nodes on the graph, 292 synthetic lethal/sick interactions, represented as edges connecting genes. All of these interactions were first identified using the automated methodology and then confirmed by tetrad analysis. To visualize sets of genes with related functions, we color-coded the genes according to their YPD cellular roles and aligned the genes based upon both their roles and connectivity. These relationships can also be represented by two-dimensional hierarchical clustering, as is used for analysis of DNA microarray experiments. For genetic interaction data, both the query genes and the interacting genes are clustered based upon the similarity of their patterns of genetic interactions (Fig. 4).

Each of the query genes were biased towards interactions with genes of particular cellular roles. Moreover, subsets of interacting genes with the same cellular roles could distinguished from one another by their connectivity. For example, the *BIM1* screen identified a large group of genes involved in mitosis (red genes), which include several components of the Bub2p- and Mad2p-dependent spindle assembly checkpoints (*BUB1*, *BUB2*, *BUB3*, *BFA1*, *MAD1*, *MAD2*, and *MAD3*) and multiple genes involved nuclear migration and spindle orientation during mitosis

(*BIK1, MCK1, SLK19, KIP3, PAC11, PAC1, NUM1, DYN1, JNM1, ARP1, ASE1*), a subset of
which also interacted with *BNI1* and function specifically as part of the Dyn1 kinesin pathway.
In addition, *BIM1* interacted with a group genes that have a chromatin/chromosome structure
cellular role (yellow genes), many of which have been implicated kinetichore function (*CTF8,*
5 *CTF19, MCM21, MCM22,* and *CHL4*), and a total of 15 genes of with unknown cellular roles
(black genes). To examine one of these uncharacterized genes in more detail, we conducted an
NBP2 screen, which showed interactions with several genes involved in nuclear migration and
spindle function (*KAR9, CIN2* and *KIP3*), actin assembly (*CAP1* and *CAP2*), and de novo
folding of actin and tubulin (*PAC10* and *GIM5*), suggestive of a general role in cytoskeletal
10 organization.

The *RAD27* screen resulted in 35 interactions, the majority of which occurred for genes
with DNA synthesis/repair cellular roles (Fig. 3 green genes). The unprocessed Okazaki
fragments of *rad27Δ* cells are probably recognized as nicks or converted into double-strand
breaks as evidenced by a large set of previously known synthetic lethal/sick interactions with the
15 genes encoding multiple components of the recombinational repair apparatus (*RAD50, RAD51,
RAD52, RAD54, RAD55, RAD57, MRE11, XRS2*) and the DNA damage checkpoint signaling
pathway (*RAD9, RAD17, RAD24, DDC1*). Intriguingly, we observed novel interactions with
HST1 and *HST2*, two genes encoding Sir3-like deacetylases and *CAC2*, a gene coding for a
chaperone that delivers acetylated histones to newly synthesized DNA, which may be indicative
20 of a functional relationship amongst the products of these genes in chromatin assembly or
silencing. *RAD27* also showed a synthetic lethal/fitness with the *SOD1* superoxide dismutase
gene, and its copper chaperone, *LYS7*, suggesting that the antioxidant functions of Sod1 are
required to protect the *rad27Δ* cells from accumulated DNA damage. Finally, *RAD27* interacted
with 4 genes of unknown function, for which we predict a possible role in a DNA synthesis
25 and/or repair.

We scored 40 synthetic lethal/sick interactions for *arc40-1*, most of which have cell
polarity or cell wall maintenance roles (Table 1), including 2 genes involved in Arp2/3 activation
(*VRP1* and *MYO5*). From a similar screen with a temperature sensitive *ARP2* allele, *arp2-1*, we
scored a total of 43 synthetic lethal/sick interactions. Strikingly, the *ARP2* screen identified a
30 large subset of 32 interactions that were shared with *ARC40*. Both screens identified a relatively

small number of unique interactions, which included genes implicated in actin patch assembly (*MYO5* with *arc40-1* but not *arp2-1*; *ARC18* and *PRK1* with *arp2-1* but not *arc40-1*) and may reflect roles specific to each Arp2/3 subunit. These findings suggest that genes whose products form functional complexes will show a highly similar but unique set of genetic interactions.

5

Example 11 - Plasmid-Borne Approaches

The pinning procedure described for the construction of double mutants provides a simple method to move a plasmid of interest into the set of approximately 5,000 viable

10   *xxxΔ::KAN* deletion mutants. In this procedure, the Y2454 starting strain is transformed with a *URA3-* or *LEU2*-based plasmid and then crossed into the haploid mutants by following steps 1-6 of the pinning procedure as described in Example 1 for genome-wide double-mutant construction. The ability to undertake plasmid-based screens with the complete deletion set greatly extends the number of possible genome-wide screens including synthetic dosage lethality

15   and green-fluorescence protein-based reporter screens.

Example 12: Synthetic Lethal/Fitness Analysis via
Bar-coded Deletion Mutants and DNA Microarray Technology

20   Genetic interactions for yeast mutant strains that contain "bar codes" for identification can be analyzed using DNA microarrays.

Step 1. The pool of *MATα xxxΔ::KAN* cells, containing the entire set of approximately 5,000 viable deletion mutants, will be mated to a Y2454-derivative for synthetic lethal analysis with a mutant allele marked with *NAT* (e.g. Y2454 made *bni1Δ::NAT*).

25

Step 2. The resultant pool of diploid cells is transferred to sporulation medium and *MATa can1Δ MFA1-HIS3* cells are selected on synthetic medium that contains canavanine but lacks histidine.

Step 3. The pool of haploid *MATa can1Δ MFA1-HIS3* cells are grown on medium containing geneticin to select for *MATa can1Δ MFA1-HIS3 xxxΔ::KAN* deletion mutants

5   **Step 4.** The *MATa can1Δ MFA1-HIS3 xxxΔ::KAN* cells are split into two samples. To determine the set of mutant cells that mated and sporulated efficiently, DNA will be prepared from one sample and used to probe the bar-coded microarray. DNA preparation of the DNA involves isolating genomic DNA and preparing bar code probes via a PCR-based method.

10   **Step 5.** To determine the set of synthetic lethal double mutants, the other sample of cells will first be grown on medium containing nourseothricin, which selects for double-mutant cells, e.g. *MATa bni1Δ::LEU2 xxxΔ::KANR can1Δ MFA1-HIS3* cells. Then, DNA will prepared for bar-coded microarray analysis. Comparison of the bar-coded mutants that are present in sample 1 but not present in sample 2 identifies potential synthetic-lethal combinations.

15

OTHER EMBODIMENTS

While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, 20   and modifications are within the scope of the following claims.